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APPENDIX

A Simplified Preparation of 2-Hydroxy-4-phosphonobutyric Acid

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2-Hydroxy-4-phosphonobutyric acid is the analogue of 3-phosphoglyceric acid in which the $-O-PO_3H_2$ group is replaced by $-CH_2-PO_3H_2$. Our previous method of making it (Dixon & Sparkes, 1974) by treatment of 2-amino-4-phosphonobutyric acid with HNO_2 had four disadvantages. These were associated with the fact that the HNO_2 was generated by addition of $NaNO_2$ to excess of HCl. Firstly, the excess of HCl slowed the reaction by overwhelming protonation of the amino groups (Taylor, 1928; Hughes *et al.*, 1958); paper electrophoresis showed that the reaction did not go to completion. Secondly, two products were formed; the unwanted one, possibly the chloro acid, had to be converted into the wanted one by boiling with alkali and the excess of alkali had then to be removed. Thirdly, evaporation of a solution of the product as the free acid in the presence of HCl gave an intractable glass, which only partly redissolved when neutralized with cyclohexylamine. Finally, the yield proved to be variable. All these difficulties are avoided in the following procedure, in which the substrate itself provides the acid necessary to convert $NaNO_2$ into HNO_2:

$$HPO_3^{-}-CH_2-CH_2-CH(-NH_3^{+})-CO_2H + NO_2^{-} \rightarrow HPO_3^{-}-CH_2-CH_2-CHOH-CO_2H + N_2$$

Method

DL-2-Amino-4-phosphonobutyric acid (Dixon & Sparkes, 1974) (4.6g) was suspended in water (300ml) and cooled to 10°C. A solution of $NaNO_2$ (7g in 20ml of water, about 4mol/mol of substrate) was added slowly with stirring. The substrate dissolved in about 10min and the solution was stirred at 20°C for 2h. Paper electrophoresis showed that the reaction was almost complete. Excess of the acid form of a sulphonic resin (Zerolit 225 SRC 14) was added and stirred with warming to 50°C until effervescence ceased (about 2h). The suspension was submitted to reduced pressure to remove dissolved N_2, and filtered through a bed (10cm × 3cm) of the same resin. The bed was washed with water. The effluent was evaporated to dryness to remove residual oxides of nitrogen, was redissolved in water, and was adjusted to pH6.5 with cyclohexylamine. On evaporation to dryness, addition of ethanol and re-evaporation, the product solidified. It was crystallized as described previously, i.e. by dissolving in methanol (150ml) and adding diethyl ether (200ml); yield 7.2g (70%).

Characterization

Elementary analysis gave: C, 49.3; H, 9.0; N, 7.3; P, 8.3% (Calc. for $C_4H_9O_6P,2C_6H_{13}N$: C, 50.3; H, 9.2; N, 7.3; P, 8.1%). The product possessed the same electrophoretic properties as the material prepared by the previous method, and acted as a substrate for 3-phosphoglycerate kinase, as described in the main paper (Webster *et al.*, 1976).

Conclusion

The modified procedure is much less laborious and gives a consistently good yield.

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